Hemoglobin

Introduction

Hemoglobin is how oxygen is transported throughout our body. Hemoglobin is found within red blood cells, the oxygen-transporting cells of the body. Red blood cells lack a nucleus and mitochondria to make room for more hemoglobin. The absence of mitochondria also ensures the absence of oxidative phosphorylation (which would use the oxygen the red blood cell is supposed deliver). Hemoglobin has a complex physiology, which is the bulk of this lesson—understanding the oxygen dissociation curve and its shifts, and how and why hemoglobin does what it does. But we start simple, building hemoglobin from its constituent parts, covering how globin determines the type of hemoglobin, and where those globin genes are expressed. We then move into a simplified version of heme synthesis.

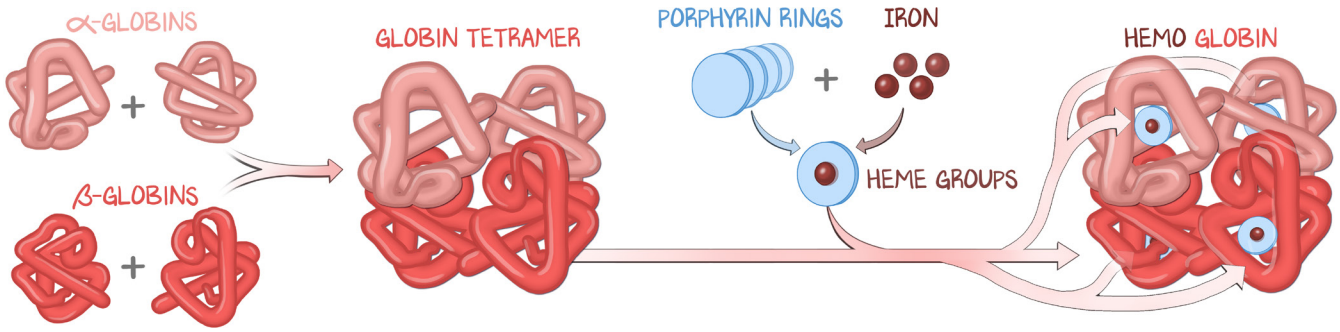

Figure 1.1: The Components of Hemoglobin
Pairs of globins form the tetramers, while porphyrin rings are loaded with iron to form heme. Each globin of the globin tetramer is associated with one heme. Hemoglobin is the combination of the globin tetramer and its associated heme molecules. Each heme, each iron, can hold one oxygen.

Globin

Heme-o-globin is a combination of four globins that form a tetramer, each globin in the tetramer associated with one heme molecule. A heme molecule is the combination of the porphyrin ring and an iron molecule. One heme molecule can bind one oxygen. There is not much you need to know about globin synthesis or degradation. Hemoglobinopathies result not from synthesis or metabolism of globin proteins, but rather from inherited gene defects and the subsequent consequences. These are discussed in Anemia #5: *Microcytic Anemia* and Anemia #7: *Normocytic Anemia*. In this section, we are going to focus on the location of globin synthesis and which globins are synthesized and when, during fetal development and after birth.

All hemoglobin is made with two α-globins. Two α-globins combine with two of any other type of globin. Hemoglobin is always two α-globins and two of any other type of globin. During initial embryogenesis, hemoglobin is synthesized from the yolk sac, and has ζ- and ε-globins, which we will not mention again. **Adult hemoglobin** is mostly hemoglobin A1 (HbA1), the combination of two α-globins and two β-globins. Adult hemoglobin also has a smidgen of hemoglobin A2 (HbA2), made of two α-globins and two δ-globins. Adult hemoglobin is made by the bone marrow. From **6 weeks' gestation to birth**, the primary source of hematopoiesis is the **liver** (with a little help from the spleen from 6 weeks to 28 weeks). The liver and spleen make **fetal hemoglobin** (HbF), the combination of two α-globins and two γ-globins. All hematopoietic centers retain the ability to make both fetal and adult hemoglobin.

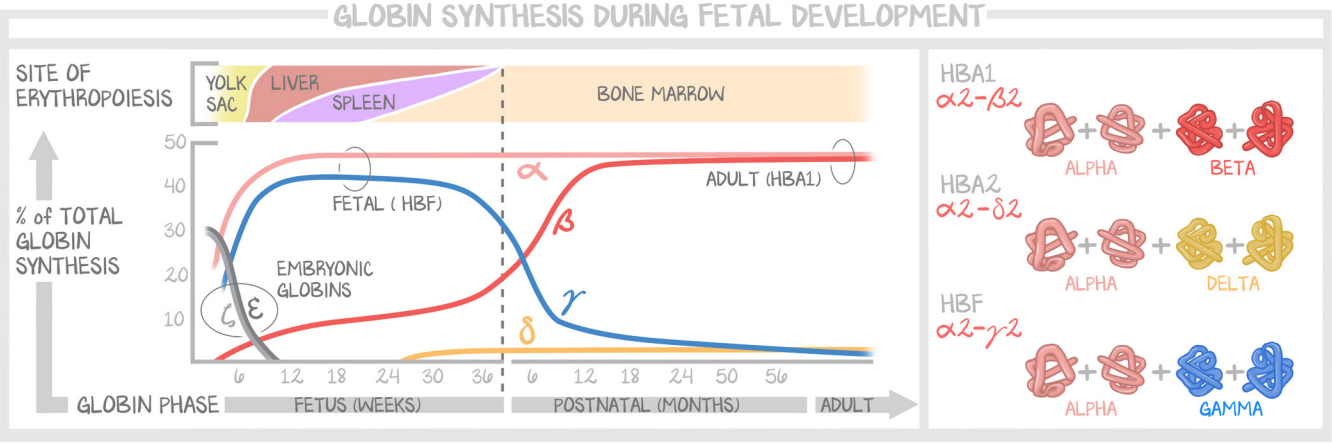

Figure 1.2: Globin Synthesis during Fetal Development and Beyond
ζ (zeta) and ε (epsilon) globins come from the yolk sac, and are of minimal impact on human medicine. α-globins are produced throughout all life, embryonic and adult, and are required for life. Initially, fetal hemoglobin is formed by the combination of α-globin and γ-globin, both of which are first made from the liver and spleen. As the marrow takes over nearing birth, it too continues to make γ-globin, but tapers off as the marrow transitions to the adult hemoglobin. Throughout most of life, adult hemoglobin, HbA1, is made by the combination of α-globins with β-globins. A trace amount of HbA2 is made by combining α-globins with δ-globins.

The significance of this comes when there is severe deficiency in the production of hemoglobin, as in severe forms of thalassemia or chronic hemolytic anemia. The organs that once could produce red blood cells may be summoned to do so again. Extramedullary hematopoiesis (the making of red blood cells somewhere other than in the red marrow of long bones)—the liver generating red blood cells, or the spleen making red blood cells—can result in elongation of non-long bones. As the bone marrow matures, it begins to take over from the liver (sometime in the third trimester).

α-globin is always expressed throughout all life. α-globin, the protein, is coded for by α-globin genes. There are **four α-globin genes** in total, two on each **chromosome 16.** Initially, the bone marrow synthesizes γ-globins, which pair to make HbF. After **birth**, the bone marrow switches from γ-globin to **β**-globin. By the sixth month of life, HbF is undetectable. β-globin, the protein, is coded for by β-globin genes. There are **two β-globin genes** in total, one on each **chromosome 11**. The pairing of two α-globins with two β-globins makes HbA1. A teeny amount of δ-globin is synthesized as well, making HbA2.

Heme—the porphyrin ring with an iron—never changes through development. Heme always associates with one globin. The type of globin chain that is paired with α-globin changes the function of the hemoglobin molecule, as we will discuss later in this lesson.

Heme

Do not. Under any circumstances. Memorize the heme synthesis metabolic pathway. We show it to you in the figure that follows as a reference only. Here's why.

The part of heme synthesis that occurs in the cytoplasm has really-hard-to-say intermediates, making it daunting. But the good news? The diseases that occur from defects in the cytoplasmic part of heme synthesis are defects of enzymes that are named incredibly well. If you memorize the name of the deficient enzyme, you immediately know the preceding intermediate, and therefore what compound accumulates.

The part of heme synthesis that occurs in the mitochondrion has not-so-hard-sounding names, and is where the rate-limiting step in heme synthesis is. The mitochondrion is where you should focus your studying. Glycine and succinyl-CoA become δ-aminolevulinic acid (henceforth, "d-ALA") by the enzyme **d-ALA synthetase**, the rate-limiting step in heme synthesis. From there, cytoplasmic stuff happens, stuff that you will only study in the context of diseases caused by heme synthesis dysfunction, which yields **protoporphyrin** back to the mitochondria. **Iron** is added to the protoporphyrin ring, becoming the iron-in-porphyrin ring, thereby completing the molecule **heme**. Iron is added to the porphyrin ring via the enzyme **ferrochelatase** (iron-chelator enzyme).

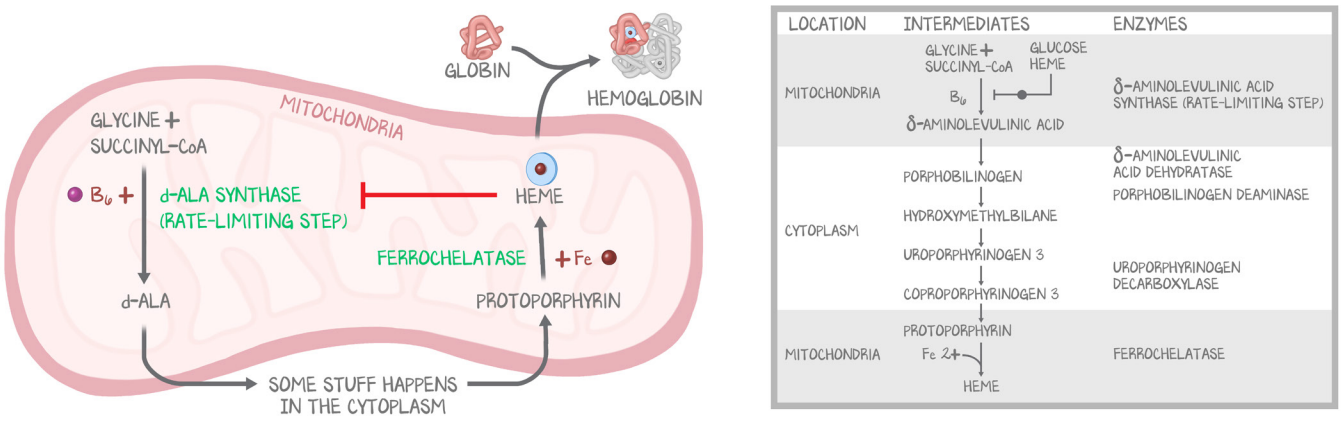

Figure 1.3: Heme Synthesis
Left Side: How we think you should memorize heme synthesis. d-ALA synthase makes d-ALA, then some stuff happens in the cytoplasm, then protoporphyrin has an iron molecule added to it to make heme, under the influence of the enzyme ferrochelatase. The heme molecule is released into the cytoplasm to make hemoglobin. Heme and glucose inhibit the rate-limiting step, the first enzyme, d-ALA synthase. *Right Side:* The entire pathway, for reference, which we actively discourage you from attempting to memorize.

The regulation of the system rests solely on the rate-limiting step, a negative feedback loop onto d-ALA synthetase. **Heme**, the product of the pathway, inhibits the pathway by inhibiting d-ALA synthase. If you have the thing you want, make less of it. If you lack the thing you want, make more. **Glucose** also inhibits the pathway, and serves as a therapeutic intervention we'll discuss in the next lesson (Anemia #2: *Disorders of Heme Synthesis*).

Hemoglobin is used by red blood cells. Red blood cells do not have a nucleus or mitochondria. Heme is synthesized in the mitochondria. Erythrocyte precursors come from a stem cell with a nucleus and mitochondria. All the hemoglobin an RBC is going to have for its entire life is synthesized in mitochondria the RBC will lose before reaching terminal differentiation.

Hemoglobin

If we take the globin tetramer and add a heme molecule (porphyrin ring with iron) to each globin, we get hemoglobin. Each heme molecule can carry one oxygen molecule. Which means that because there are four globins, and therefore four hemes, one molecule of hemoglobin can carry up to four oxygen molecules.

Hemoglobin demonstrates a property called **positive cooperativity**. The fact that there are four globin molecules means that they can shift relative to each other; the hemoglobin molecule can undergo conformational changes. These conformational changes within the tetramer **alter the affinity of the heme molecules for oxygen**. The thing that causes the conformational change is the binding of oxygen to one

heme molecule. As oxygen is added to heme molecules, the other hemes of that hemoglobin are more likely to bind oxygen. As oxygen binds, the affinity for oxygen increases, making more oxygen binding more likely. Conversely, as more oxygen is unloaded, the affinity for oxygen decreases, making oxygen unloading more likely. If that doesn't immediately jive, that's okay. Let's take a look at why it would do that.

Oxygen is a molecule and obeys the laws of diffusion. Oxygen will go from a region of high oxygen to low oxygen, flowing down its concentration gradient. But that happens at a microscopic level. Oxygen can't diffuse from your lungs to your toes by itself. That's why we have red blood cells and a cardiovascular system. We use local diffusion at the lung to load oxygen onto hemoglobin, then we use local diffusion at the tissues to unload oxygen from hemoglobin. In between, the hemoglobin (in RBCs) is pushed through the tubing we call blood vessels, propelled by the contraction of the heart.

Let's start at the lungs. Oxygen diffuses from the lungs, the alveoli, across the alveolar capillary, and onto a heme molecule of hemoglobin in an RBC flowing through the pulmonary capillary. RBCs have to deform to fit through the capillaries, bringing their plasma membrane in contact with the plasma membrane of the endothelial cell that lines the capillary, which in turn is in contact with the plasma membrane of the alveolus. This makes for the smallest possible diffusion barrier. Within the alveolus is oxygen. A lot of oxygen. The driving force for oxygen to move from the alveolus to the RBC is massive, and the diffusion barrier small. Oxygen moves into the RBC and therefore onto hemoglobin. In this environment, the only source of oxygen for the entire body, the only thing that should happen is oxygen is loaded onto hemoglobin. "A lot of oxygen" means a high partial pressure of oxygen. At high partial pressures, oxygen diffuses onto and binds hemoglobin. As oxygen binds to hemoglobin, positive cooperativity makes oxygen more likely to bind hemoglobin. Therefore, in the lungs, aka, **at high partial pressures of oxygen, positive cooperativity provides a feedforward mechanism that ensures maximum uptake of oxygen onto hemoglobin**. In the most favorable position, the most likely to bind oxygen, the tetramer is said to be in the **relaxed state** (*relaxed for respiratory*). The partial pressure of oxygen is 100 mmHg at the alveolus.

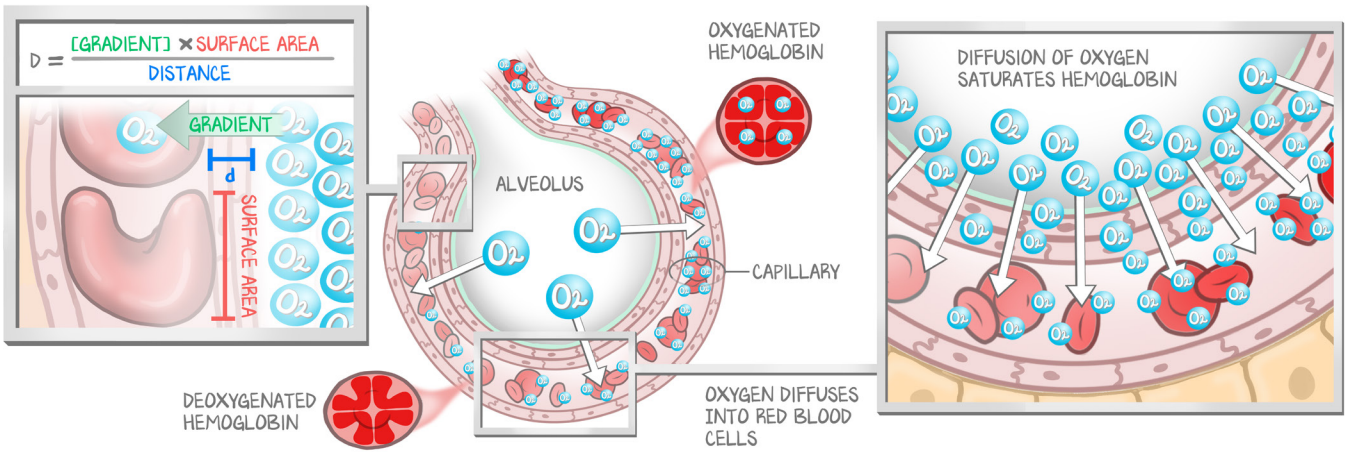

Figure 1.4: Hemoglobin in the Lungs
The capillaries' small size ensures a minimal diffusion barrier at the alveolar-capillary exchange. The lungs have high oxygen tension, greatly facilitating oxygen diffusion onto hemoglobin via a strong concentration gradient. The conformational change to the relaxed form further facilitates oxygen uptake.

RBCs are circulated through the arterial system by the heart. See the RBCs as just drifting along through the pipes, plump sacs of oxygen bound to hemoglobin. Suddenly, the RBC meets another capillary—the capillary of any tissue other than the lung. This one happens to be the capillary of an active skeletal muscle. The partial pressure here is 15 mmHg (this is an extreme example used to illustrate the conformational change; we will discuss resting capillaries next).

The RBC is in a capillary, and so deforms, bringing its plasma membrane into contact with the plasma membrane of the endothelial cell that lines the capillary, which in turn is in contact with the plasma membrane of the skeletal muscle fiber (the sarcolemma). The diffusion barrier is small. There is a lot of oxygen on the hemoglobin in the RBC. There is not a lot of oxygen in the tissues. Oxygen diffuses from a region of higher concentration to a lower concentration—off the hemoglobin and into the tissues. As oxygen unloads, positive cooperativity results in a conformational change AWAY from the state that was in the lung, making oxygen UNloading more likely. As oxygen unloads, flowing down the concentration gradient, the tetramer continues to change shape. The more it changes shape, the more likely oxygen is to unload. Therefore, akin to the reverse of the lungs, **at low partial pressures of oxygen, positive cooperativity provides a feedforward mechanism that ensures maximum unloading of oxygen from hemoglobin to the tissues**. In the most favorable unloading position, the most likely to unload oxygen, the tetramer is said to be in the **taut state** (*taut for tissues*).

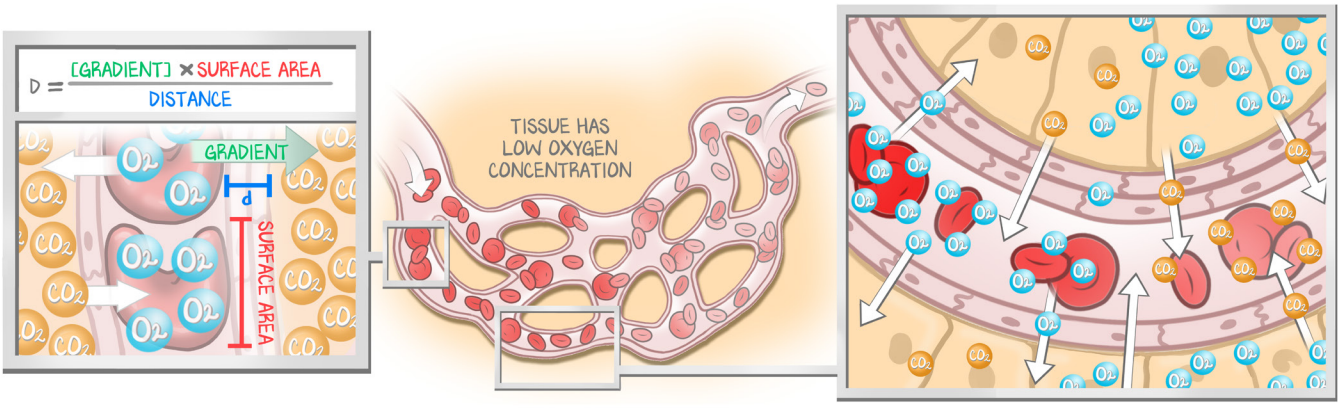

Figure 1.5: Hemoglobin in the Tissues
The capillaries' small size again minimizes the barrier to diffusion of oxygen. Active tissues have a low oxygen tension, facilitating diffusion of oxygen off of hemoglobin via the favorable concentration gradient of oxygen from RBCs to the tissue, unloading oxygen into the tissues. This unloading favors the taut state, further unloading oxygen.

We used the directionality and word choices on purpose. In the lungs, hemoglobin takes up oxygen, and is in the most favorable position to take up oxygen. In the tissues, hemoglobin unloads oxygen, and is in the most favorable position to unload oxygen. "The most favorable position to unload oxygen" is synonymous with "the least favorable position to take up oxygen." To avoid confusing you with flipped arrows, we chose words that didn't sound alike (take up, unload), and discussed the conformation change from the perspective of what the we want the RBC to do in each location.

In other words, if oxygen is high, hemoglobin binds oxygen and assumes a shape that makes it better at binding more oxygen. If oxygen is low, hemoglobin releases oxygen and assumes a shape that makes it better at releasing more oxygen. The tissue oxygen concentration tells hemoglobin what to do. This defines the ends of the oxygen dissociation curve—the extreme taut and relaxed forms. Next, we discuss what happens in the middle.

Oxygen Dissociation Curve

The partial pressure of oxygen in capillaries of tissue at rest is about 40 mmHg. At this partial pressure of oxygen, most oxygen remains bound to hemoglobin. A little oxygen comes off, but most stays bound to hemoglobin. This is okay, as the system is set up to carry a redundant amount of oxygen for tissues at rest. It wouldn't be very smart to have a system that completely drained all the oxygen all the time—in times of stress, there would be no backup. Other signals can lead to vasodilation (Cardiology), which brings more blood to the tissue. More blood means more hemoglobin. More hemoglobin means more oxygen. But the hemoglobin system alone has redundancy.

The P_{50}, the partial pressure of oxygen at which hemoglobin is half saturated, is about 27 mmHg. Teleologically, we want a molecule that is really good at taking up oxygen in the lungs and really good at giving oxygen to tissues that need it. We also want that molecule to act as a reservoir, only giving excess oxygen when the tissue really needs it. That means having a wide range of oxygen partial pressures where not much changes. That is what gives us the **sigmoid shape** of the oxygen dissociation curve. The sigmoid shape is a product of cooperativity.

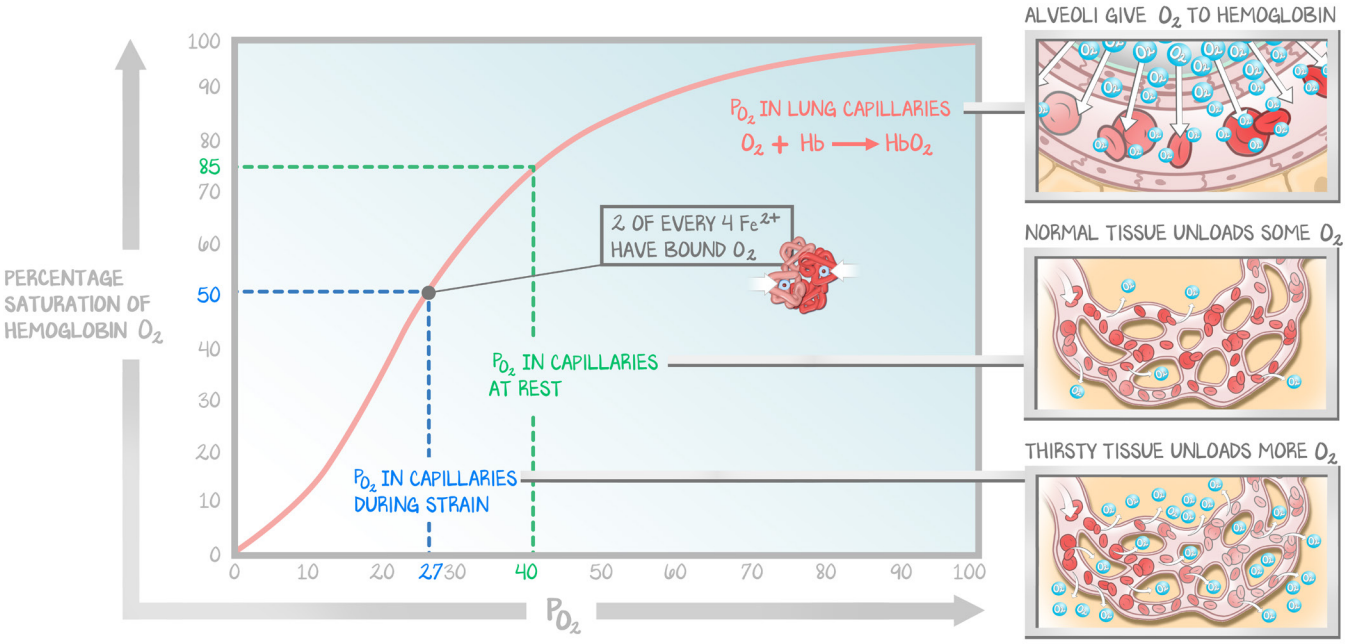

Figure 1.6: The Oxygen Dissociation Curve
The curve is flat from 60–100 mmHg, meaning humans can tolerate wide shifts in atmospheric conditions and still manage to saturate all hemoglobins. By binding hemoglobin at high oxygen tensions, the partial pressure of free oxygen is kept low, facilitating diffusion. The steep range of the curve ensures that all tissues receive oxygen, but also ensures redundancy in oxygen transport—most of the oxygen is still bound in mixed venous blood. At the low end of the curve, that steep decline ensures that once a low PaO_2 is reached, oxygen vigorously unloads into the tissues.

Shifting the Curve

So far, we've discussed hemoglobin's affinity for oxygen relative to the oxygen concentration. Oxygen alone has been the signal. When more oxygen is around, more oxygen binds, the conformational shape trends toward relaxed, and therefore more oxygen binds. When less oxygen is around, more oxygen unloads, and the conformational shape trends toward taut, therefore unloading more oxygen. This is what gave us the sigmoid curve. Tissues that are active consume oxygen, and low oxygen signals hemoglobin to release oxygen.

But the tissues can use more than just oxygen concentrations to signal hemoglobin to release oxygen. Signs of increased metabolic activity can signal the need for more oxygen. Increased metabolic activity means more glycolysis-TCA-electron transport chain. To do oxidative phosphorylation (emphasis on oxidative), tissues use oxygen. Oxygen levels fall. Hemoglobin assumes the conformational shape to unload oxygen. But when tissues use oxygen to do glycolysis-TCA-electron transport chain, other molecules are generated. Glycolysis produces **2,3-BPG**. Oxidative phosphorylation makes the product of cellular respiration, **carbon dioxide**. And if there isn't enough oxygen around to do oxidative phosphorylation, anaerobic metabolism generates **lactic acid**, reducing the pH of the tissue. Metabolic activity generates heat, **elevating temperature.** Not surprising, then, the situations that make oxygen more likely to fall off hemoglobin are the presence of 2,3-BPG and CO_2, low pH, and high temperature.

What confuses students is the biochemistry behind this, the **right shift** of the oxygen dissociation curve. In biochemistry and general pharmacology we had enzyme curves, percent efficacy on the y-axis and K_M on the x-axis. Remember those? We defined a higher affinity by a lower K_M (to the left on the curve) and a lower affinity by a higher K_M (to the right on the curve). What did we just do to the oxygen dissociation curve? Shifted it to the right. And if hemoglobin were an enzyme, with 100% efficacy defined by 100% saturation with oxygen, by shifting to the right, did we change the V_{max}? We did not. One hundred percent saturation remains the "V_{max}" of the curve. But there would need to be more substrate (oxygen) around to achieve 100% saturation. Why is that? Because a shift to the right means a higher K_M, and therefore a lower affinity for its substrate (oxygen). What does a lower affinity for oxygen mean? It means more likely to unload oxygen, more likely to assume the taut conformational shape. As soon as the RBC moves through the capillary and away from the region with the CO_2, 2,3-BPG, and low pH, the signal to shift right is removed, and back to normal it goes.

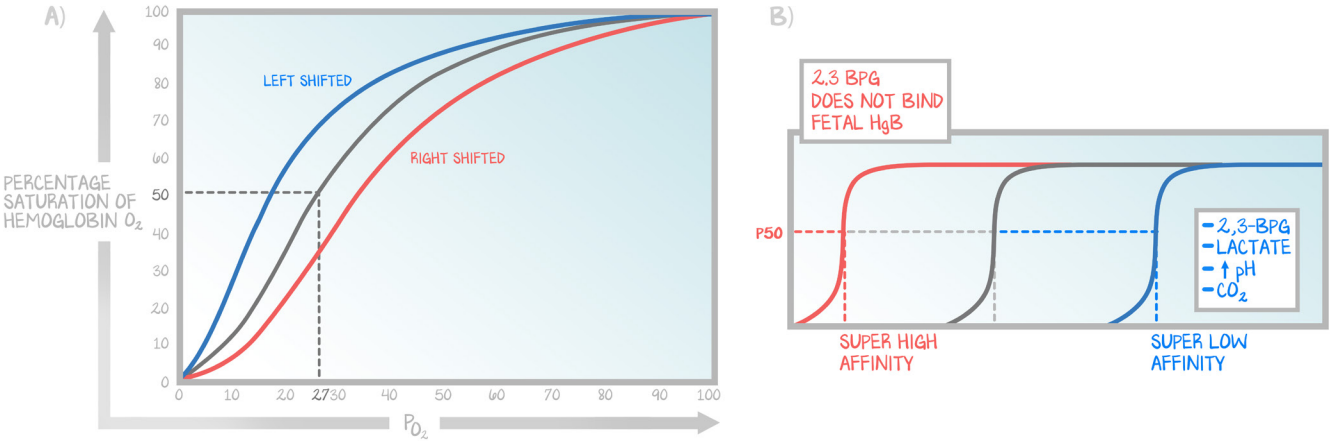

Figure 1.7: Shifting the Oxygen Dissociation Curves
(a) What the curve looks like in real life. When right shifted, the P_{50} is higher—oxygen is less likely to be bound to hemoglobin. When left shifted, the P_{50} is lower—oxygen is more likely to be bound to hemoglobin. This illustration is accurate, but can be confusing. (b) An exaggerated version of the same curve, drawn to appear familiar to an OME student who has been through our Biochemistry and The Cell modules. The exaggerated form is used to relate to knowledge already acquired with regard to enzyme kinetics to explain the left and right shifts. The blue line is shifted to the right, therefore has a lower affinity. The red line is shifted to the left, therefore has a higher affinity. The "V_{max}" is the same—all hemoglobins can fully saturate with enough oxygen around, it's just whether this molecule is more or less likely to unload oxygen (lower affinity, more unloading).

A left shift would increase hemoglobin's affinity for oxygen, making it more likely to bind. The only meaningful **left shift** is **fetal hemoglobin**, which has a higher affinity for oxygen at all times because it **ignores the 2,3-BPG signal**. The other "left shifts" are actually more akin to "undoing the right shift," as in restoration of metabolic activity resulting in normalization of CO_2, pH, and 2,3-BPG.

Calling on Pulmonary Knowledge

It is expected that you already have intimate knowledge of the delivery of oxygen equation, and the role hemoglobin will play. Likewise, you are expected already to know concepts such as the Bohr effect, the relationship of O_2 to CO_2 and nitrogen, the FiO_2 in the air, etc. This Anemia series focuses on the heme and globin components of oxygen delivery, and assumes oxygen is in the lungs and is needed at the tissues.

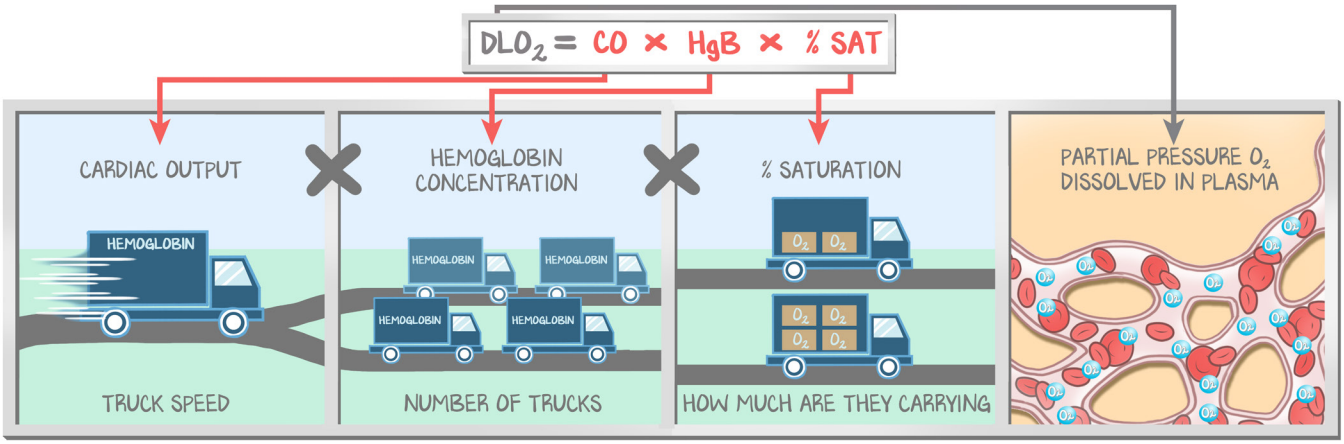

Figure 1.8: Delivery of Oxygen Equation
The delivery of oxygen has three components. The first is the cardiac output, covered in Cardiology, and is how fast the trucks can drive. The second is the hemoglobin concentration, how many trucks are available. The third is the percent saturation, how much oxygen is on each truck. There is a fourth component to the delivery of oxygen, which is the partial pressure of oxygen dissolved in plasma. This number represents an extremely small part of oxygen delivery because the coefficient is such a small number, though it can play a role in management of hypoxemic disease.